Inulin Hydrogels as Carriers for Colonic Drug Targeting: I. Synthesis and Characterization of Methacrylated Inulin and Hydrogel Formation

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Purpose. Vinyl groups were introduced in inulin chains in order to form hydrogels of this sugar polymer by free radical polymerization.
Methods. Inulin was reacted with glycidyl methacrylate in N,N-dimethylformamide in the presence of 4-dimethylaminopyridine as catalyst. ^{1}H and ^{13}C NMR spectroscopy were used for the characterization of the obtained reaction product. Solid state ^{13}C NMR spectroscopy revealed the conversion of the incorporated vinyl groups into covalent cross-links upon free radical polymerization of aqueous solutions of the derivatized inulin.
Results. During reaction of inulin with glycidyl methacrylate, transesterification occurred, leading to the direct attachment of the methacryloyl group to inulin. Consequently, the obtained reaction product is methacrylated inulin. The extent of chemical modification of inulin could be tuned by varying the molar ratio of glycidyl methacrylate to inulin in the reaction mixture. Aqueous solutions of methacrylated inulin were converted into cross-linked hydrogels by free radical polymerization using ammonium persulphate and N,N,N′,N′-tetramethylethylenediamine as initiating system.
Conclusions. Inulin hydrogels can be formed by free radical polymerization of aqueous solutions of methacrylated inulin.

KEY WORDS: inulin; glycidyl methacrylate; hydrogels; NMR spectroscopy.

INTRODUCTION

Inulin is a naturally-occurring storage polysaccharide found in many plants such as onion, garlic, artichoke and chicory (1). Chemically, it belongs to the glucofructans. It consists of a mixture of oligomers and polymers containing 2 to 60 (or more) β 2-1 linked D-fructose molecules. Most of these fructose chains have a glucose unit as the initial moiety. Inulin is considered a dietary fiber, meaning that it is not hydrolyzed by the endogenous secretion of the human digestive tract (2). However, bacteria residing in the colon, and more specifically *Bifidobacteria,* are able to ferment inulin (3,4,5). *Bifidobacteria* constitute up to 25% of the normal gut flora of both man and animals (6). This bifidogenic effect of inulin and the related health promoting properties (7) are already commercially exploited. Therefore, it is interesting to explore whether inulin has potential as a new carrier for colonic drug targeting.

Approaches to obtain colon-specific drug delivery based on bacterially-degradable polymers, comprise degradable prodrugs (8), degradable coatings of sugar polymers (9) or azo bonds containing polymers (10), or degradable matrices (11) and hydrogels (12,13). In previous work, inulin suspension films in Eudragit RS were prepared. Degradation of the films by fecal bacteria was demonstrated, but it was not very reproducible (14). This paper describes the development of inulin hydrogels, the first part in the study of the potential of inulin hydrogels as carriers for colonic drug targeting. Inulin is derivatized with glycidyl methacrylate to introduce vinyl groups in the fructose chains which can be converted in a next step into cross-linked hydrogels by free radical polymerization of aqueous solutions of the derivatized inulin. Thorough characterization of the derivatized inulin led to new conclusions compared with literature data.

MATERIALS AND METHODS

Materials

Chicory inulin (Raftiline HP; average degree of polymerization between 22 and 25) was kindly provided by Tiense Suikerraffinaderij (Tienen, Belgium). N,N-dimethylformamide (DMF) and ammonium persulphate (APS) were supplied by UCB (Leuven, Belgium). Glycidyl methacrylate (GMA;96%) and isopropanol (anhydrous, 99+%) were purchased from Acros Organics (Geel, Belgium) and 4-dimethylaminopyridine (DMAP) and N,N,N′,N′-tetramethylethylenediamine (TMEDA; 99%) were obtained from Sigma (St. Louis, USA). Cellulose ester dialysis membranes with molecular weight cut-off of 500 and Sephadex G-25 M PD-10 columns were supplied by Spectrum (CA, USA) and Pharmacia Biotech AB (Uppsala, Sweden) respectively. All other chemicals were used as received. The water used for dialysis was purified with a Milli-Q system of Millipore (Brussels, Belgium).

Synthesis of Methacrylated Inulin

For the synthesis of methacrylated inulin (MA-IN), 50 g of dried sugar was dissolved in 200 ml of DMF. DMAP was added in a concentration of 10 mol % *versus* fructose units. Depending on the desired degree of substitution (DS), *i.e.* the amount of methacryloyl groups per 100 fructose units, a calculated amount of GMA was added. After stirring the reaction mixture for 72 h at room temperature, the reaction product was precipitated and washed in isopropanol. To remove all traces of DMAP, the precipitate was subsequently dissolved in Milli-Q water and dialyzed for 10 days at 4°C against the same solvent. Prior to purification, DMAP was neutralized by adding an equimolar concentration of HCl dropwise to the reaction mixture in order to prevent alkaline hydrolysis of the methacrylic ester during dialysis. The derivatized inulin was recovered after purification by lyophilization.

The methacrylation reaction of inulin with GMA was monitored in different conditions by taking samples of the reaction mixture at regular time intervals. The sugar polymer was separated from solvent, catalyst, unreacted GMA and glycidol by

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eluting the sample over a Sephadex G-25 M PD-10 column. The front fractions were lyophilized and the DS was determined by ^{1}H NMR spectroscopy.

NMR Spectroscopy

To characterize the derivatized inulin and to determine the obtained DS, ^{1}H and ^{13}C NMR spectra were recorded. ^{1}H NMR spectra were recorded in D_2O (30 mg in 0.7 ml) with a Gemini 200 MHz spectrometer (Varian, CA, USA). The water signal, used as reference line, was set at 4.80 ppm and was suppressed by irradiation during the relaxation delay. A pulse angle of 90° was used and the relaxation delay was 30 s (at least five times the longest T_1 of the peaks of interest, as measured in preliminary T_1 experiments). To have a good S/N ratio for integration, the number of scans was 16. ^{13}C NMR spectra were recorded in dimethylsulfoxide-d_6 (60 mg in 0.6 ml) with a Bruker AMX 400 spectrometer (Bruker, Karlsruhe, Germany). The septet of the solvent signal was used as internal reference and placed at δ 39.5 ppm versus tetramethylsilane. A pulse angle of 30° and a relaxation delay of 1 s was used. The DEPT 135 pulse sequence was used to differentiate between CH, CH_2 and CH_3 type of carbons. The number of scans was 16,000 for ^{13}C and 4000 for DEPT 135 NMR spectroscopy.

Synthesis of Glyceryl Monomethacrylate

Synthesis of glyceryl monomethacrylate was performed as follows (15): 10 g of GMA was added to 16 ml of demineralized water containing 0.04 ml of concentrated sulfuric acid. To prevent polymerization, a small additional amount of hydroquinone was added. The reaction mixture was stirred for 7 days at room temperature. After neutralization with 0.1 M NaOH and saturation with NaCl, it was extracted with dichloromethane. The obtained extract was subsequently dried over magnesium sulphate, filtered, concentrated under reduced pressure and purified by column chromatography on silica gel (13 cm × 2 cm) by eluting with $CHCl_3$ and $CHCl_3$: MeOH (90:10) resulting in a colorless, viscous liquid (yield : 43.4% based on the theoretical obtainable amount). The structure was proven by ^{13}C NMR spectroscopy (Fig. 3B).

Determination of the DS by Titration

In addition to the calculations based on the ^{1}H NMR spectra, the DS of the derivatized inulin was also determined by titration. MA-IN (50 mg) was dissolved in 0.1 N NaOH (4 ml) and stirred for 72 h to obtain alkaline hydrolysis of the methacrylic ester. The molar consumption of NaOH was determined by back titration with 0.1 N HCl after adding 3 drops of phenolphthalein solution (16) as indicator. Underivatized inulin was used as blank. The DS was calculated using the following equation:

$$\frac{Mol_{NAOH} \times MW_F}{W_p - Mol_{NAOH} \times MW_{MA}} \times 100 \qquad (1)$$

where Mol_{NaOH} is the molar consumption of NaOH, MW_F is the molar weight of a fructosyl unit −1, W_p is the weight of MA-IN powder that was hydrolyzed, MW_{MA} is the molar weight of the methacryloyl group.

Carbohydrate Analysis

Carbohydrate analysis of 0.05% solutions of inulin and MA-IN was performed by HPLC (Dionex, Sunnyvale, CA, USA) on a Carbopac PA1 anion-exchange column with pulsed amperometric detection. The elution conditions (17) were 90 mM NaOH with 50 mM sodium acetate for 1 min followed by a linear gradient from 50 to 500 mM sodium acetate in 90 mM NaOH over 60 min. The flow rate was 1 ml min^{-1}. After every run, the column was regenerated with 1 M NaOH for 10 min and equilibrated for 20 min to the initial conditions.

Preparation of MA-IN Hydrogels

Aqueous solutions of MA-IN were converted into cross-linked hydrogels by free radical polymerization using APS and TMEDA as initiating system (18). MA-IN (400 mg) was dissolved in 1 ml of a 0.5 M phosphate buffer pH 6.5. 17.5 μmol APS and 39.4 μmol TMEDA were added. The mixture was then divided over molds and polymerization took place at room temperature for 2.5 h resulting in cross-linked hydrogels with a diameter of 10 mm and a height of 2–3 mm.

In order to follow the conversion of the double bonds upon polymerization, FT-IR and solid state ^{13}C NMR spectroscopy were used. FT-IR spectra of KBr tablets containing MA-IN were recorded with a Perkin-Elmer System 2000 FT-IR spectrometer (Perkin-Elmer, CT, USA) between 4000 and 450 cm^{-1} with a resolution of 2 cm^{-1}. For each spectrum 24 scans were taken. The CP/MAS ^{13}C NMR spectra were obtained at 75.47 MHz on a Bruker AMX-300 spectrometer (Bruker, Karlsruhe, Germany) with a 4 mm double bearing probe head. A spinning rate of 12 kHz and spin locking and decoupling fields of approximately 50 kHz were used. The contact time was 3 ms and a recycle time of 3 s was applied. The number of scans was 9000. The samples were prepared by lyophilization of MA-IN solutions at time t = 0 h and t = 2.5 h of the polymerization process.

RESULTS AND DISCUSSION

Synthesis of Methacrylated Inulin

Derivatization of inulin with GMA was based on a method described by van Dijk-Wolthuis (15). Adaptations were intro-

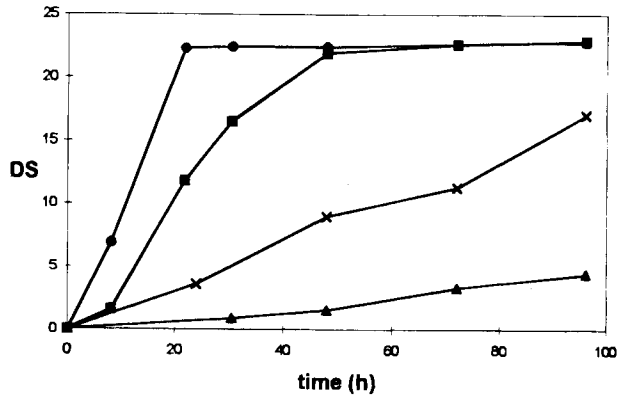

Fig. 1. Obtained DS as a function of time for the reaction of GMA with inulin (molar ratio of GMA to inulin = 0.3) in a concentration of 21% w/w in the presence of 25 mol% (●), 10 mol% (■) and 1 mol% (▲) DMAP as catalyst, and for the reaction with inulin in a concentration of 12% w/w in the presence of 10 mol% DMAP (×).

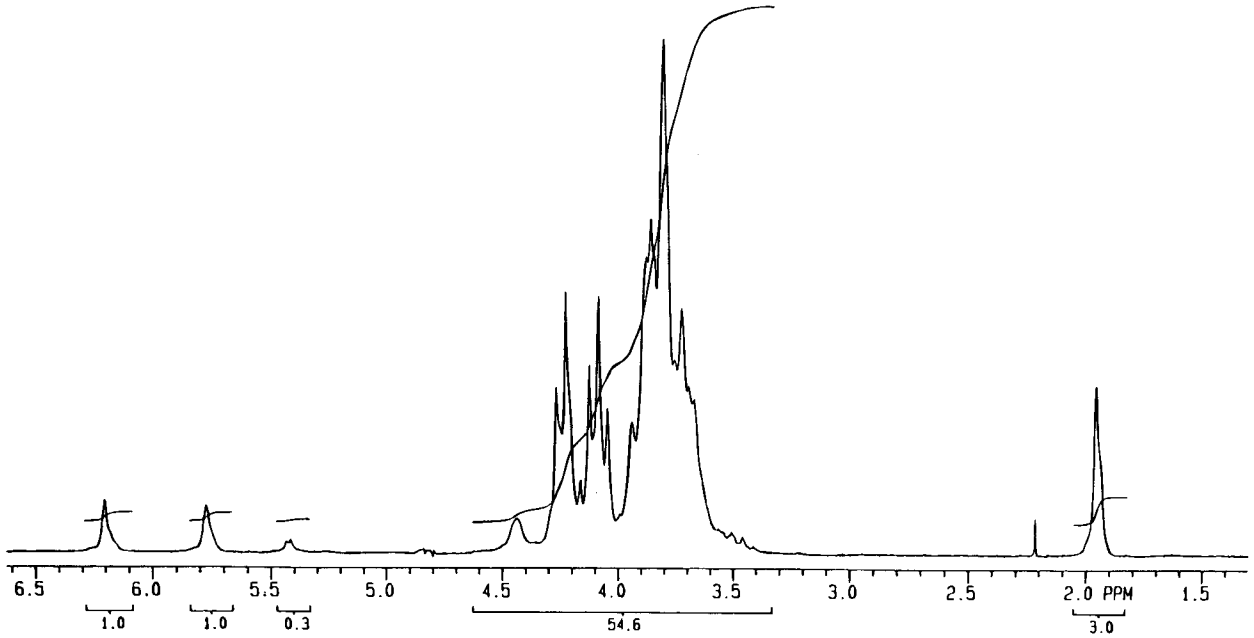

Fig. 2. ^{1}H NMR spectrum of methacrylated inulin DS=12.8.

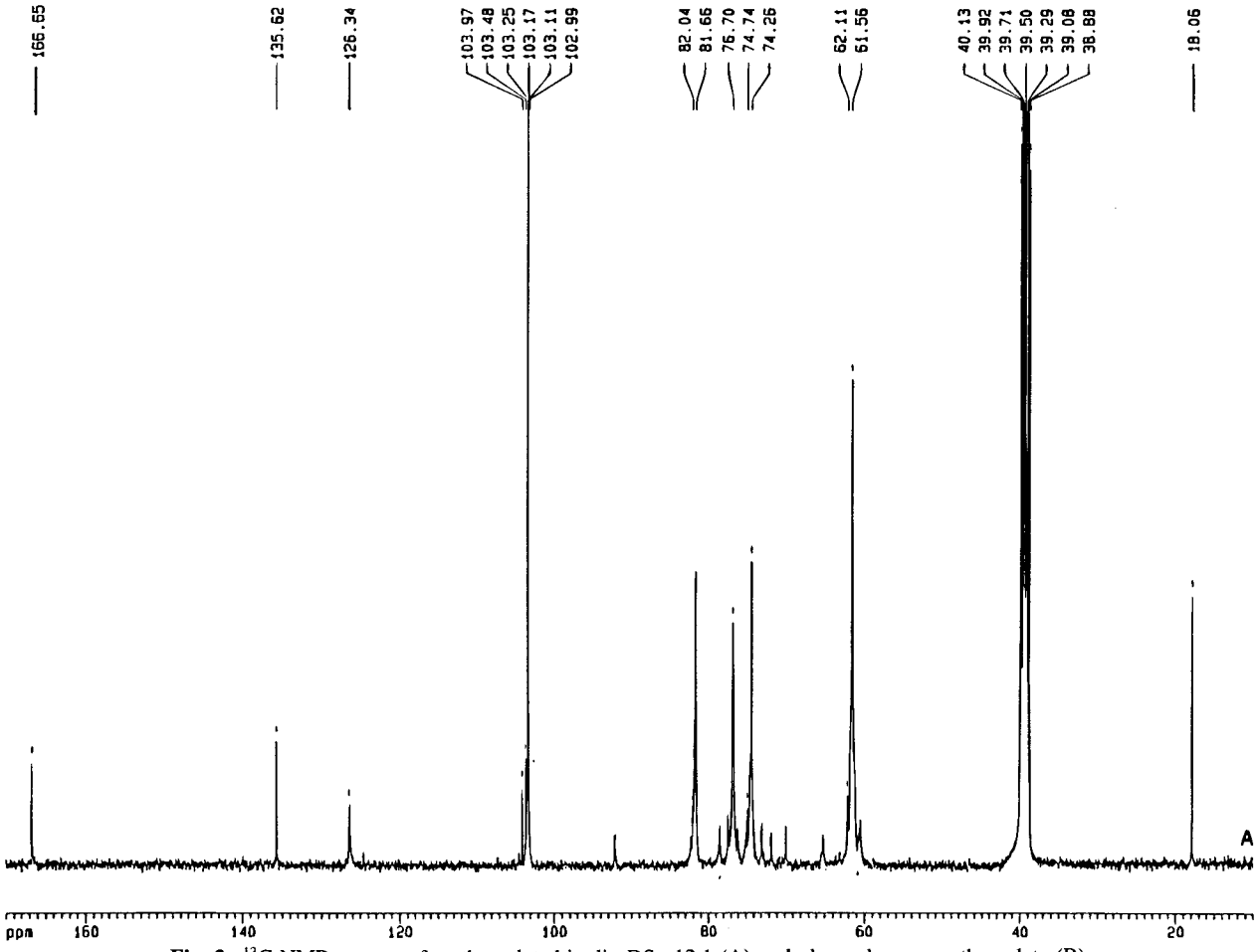

Fig. 3. ^{13}C NMR spectra of methacrylated inulin DS=12.1 (A) and glyceryl monomethacrylate (B).

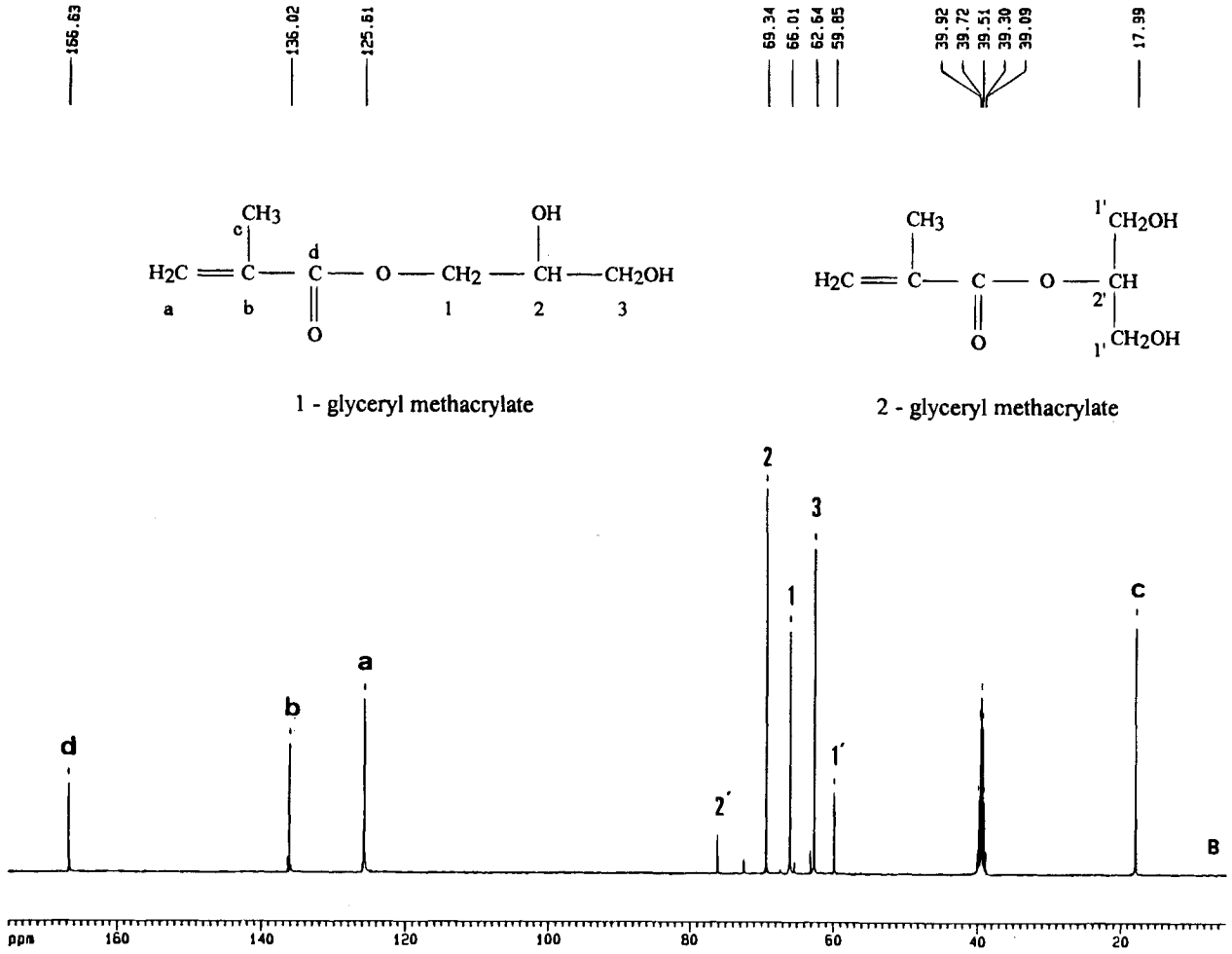

Fig. 3. Continued.

Table I. ^{13}C NMR Chemical Shifts (DMSO-d_6 at 39.5 ppm Versus TMS) of Methacrylated Inulin and the Methacrylation Shifts

Atom	^{13}C shift of main carbons of MA-IN	^{13}C shift of additional peaks of MA-IN	Methacrylation shift ($\Delta\delta$)
C-1	61.56	60.49	−1.07
C-2	103.25	103.48	+0.23
C-3	76.70	76.07	−0.63
C-4	74.26	74.97	+0.71
C-5	81.66	78.46	−3.20
C-6	61.56	65.16	+3.60
CCH_3	18.06		
$C=CH_2$	126.34		
CCH_3	135.20		
$C=O$	166.65		

Table II. Degree of Substitution (DS) of Methacrylated Inulin as Determined by ^1H NMR Spectroscopy and Titration (n=3)

Theoretical DS	Obtained DS as determined with		Average efficiency[a]
	^1H NMR	titration	
5	4.36 (±0.05)	4.53 (± 1.49)	0.89
10	8.14 (± 0.16)	8.42 (± 0.55)	0.82
15	12.13 (± 0.61)	11.88 (± 1.62)	0.80
20	15.45 (± 0.13)	15.61 (± 1.27)	0.78
30	22.33 (± 0.20)	21.24 (± 1.40)	0.72

[a] average efficiency is calculated as the ratio of obtained DS to theoretical DS.

duced, especially in the isolation/purification procedure : purification of the reaction product could not be performed by dialysis of the reaction mixture as such because of the incompatibility of the dialysis tubes and DMF, the aprotic solvent that was used. Instead, derivatized inulin was precipitated and washed in isopropanol to remove solvent, catalyst, glycidol and unreacted GMA. However, even after several cycles of dissolution, precipitation and washing, there were still traces of DMAP present (isopropanol and DMF could be evaporated under reduced pressure). Hence, the precipitated and washed MA-IN was dissolved in Milli-Q water and dialyzed for 10 days at 4°C. This resulted, after lyophilization, in a white fluffy product in which no impurities could be detected by ^1H NMR spectroscopy (yield : 30–54% based on theoretical amount of MA-IN assuming a 100% incorporation, except for DS = 22.3 where the yield was only 14%).

The extent of the methacrylation of inulin at room temperature as a function of time for different concentrations of DMAP

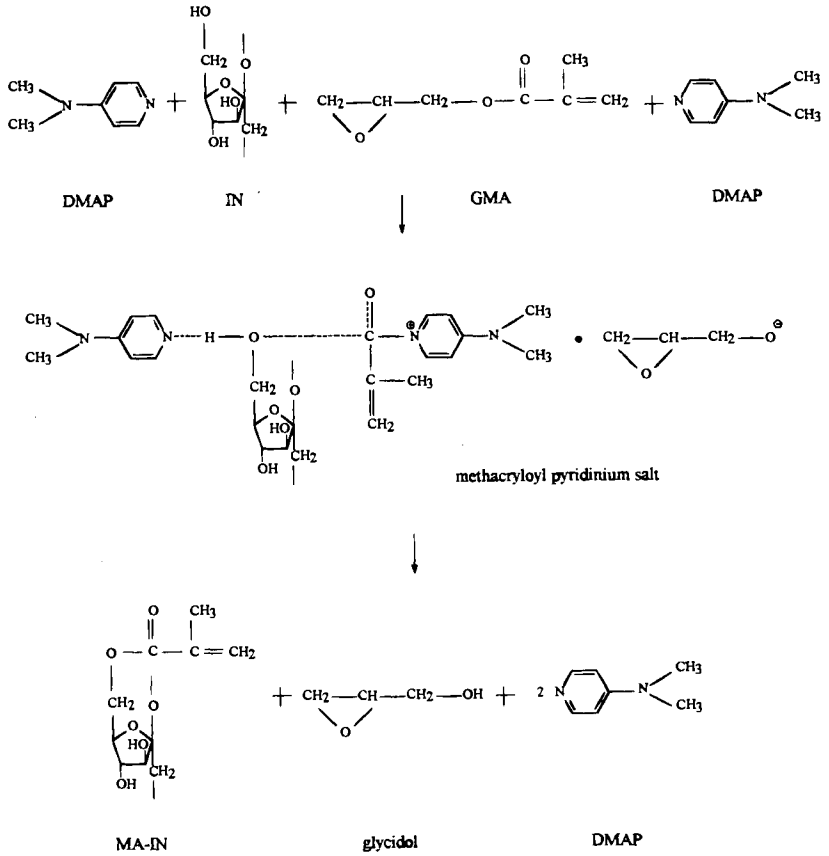

Fig. 4. Schematic representation of the proposed reaction mechanism for the reaction of inulin with GMA in the presence of DMAP as catalyst.

and for different dilutions of the reaction mixture, is shown in Fig. 1. From these results, it was concluded to perform the large scale synthesis of MA-IN with a 21% w/w concentration of inulin in DMF in the presence of 10 mol% DMAP *versus* fructose units for 72 h at room temperature. In the absence of DMAP, methacrylation of inulin did not take place.

Characterization of Methacrylated Inulin

In order to characterize the derivatized inulin, ^{1}H and ^{13}C NMR spectra were recorded. Fig. 2 shows the ^{1}H NMR spectrum of MA-IN (DS = 12.8). All sugar protons are situated between δ 3.30–4.60 ppm, except the anomeric proton, at δ 5.42 ppm, of the D-glucopyranosyl unit of the α1-β2 osidic linkage initiating the inulin chain. The signals from the methacryloyl group are observed at δ 1.97 ppm (methyl protons) as well as at δ 5.78 and δ 6.20 ppm (protons at the double bond). The ^{13}C NMR spectrum of MA-IN (DS = 12.1) is depicted in Fig. 3A and the chemical shifts are listed in Table I. Surprisingly, no signals from the glyceryl part could be detected. This indicated that the reaction did not take place at the epoxide ring, in contrast to literature data (15) where opening of the ring was suggested. Instead, transesterification occurred, resulting in the formation of methacrylated inulin instead of glyceryl methacrylated inulin. In order to position the missing carbon signals of the glyceryl part on the spectrum glyceryl monomethacrylate was synthesized. The product merely consists of 2 isomers : 1-glyceryl- and 2-glyceryl methacrylate, the former being the main product (Fig. 3B).

Signal 1 (δ 66.01 ppm) and signal 2 (δ 69.34 ppm) of 1-glyceryl methacrylate could indeed not be detected in the spectrum of MA-IN. Moreover, attachment of the glyceryl part

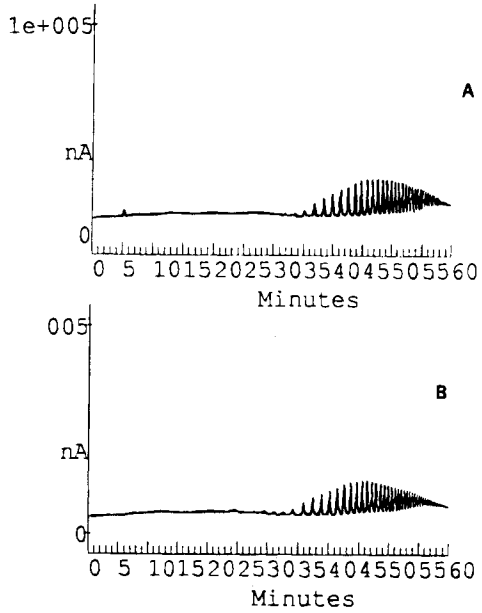

Fig. 5. HPAEC-PAD chromatographs of inulin (A) and methacrylated inulin DS=4.4 (B)

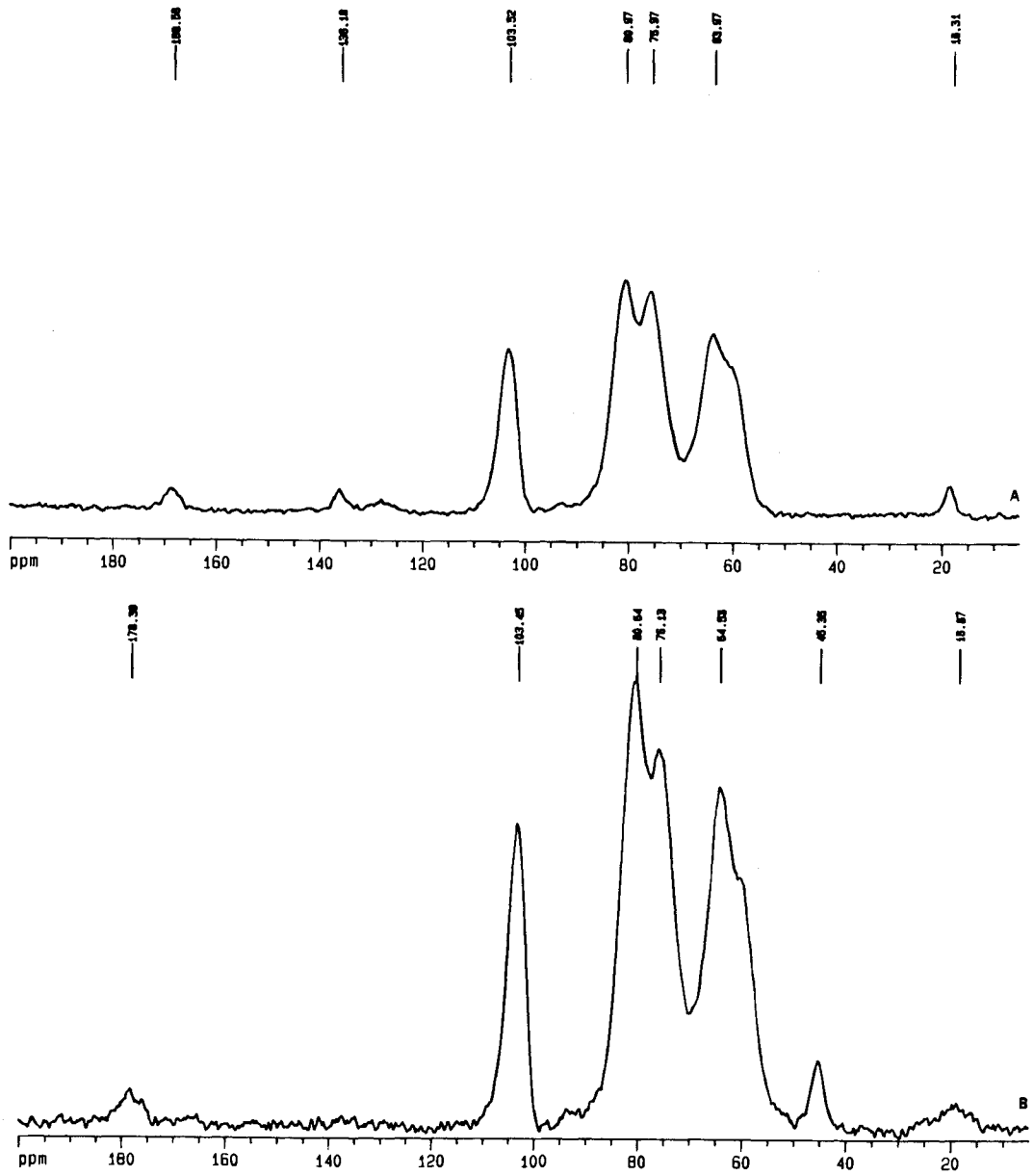

Fig. 6. Solid state CP/MAS ^{13}C NMR spectra of methacrylated inulin DS=12.1 at time t = 0 h (A) and t = 2.5 h (B) of the polymerization reaction.

of GMA to inulin should also result in a methylene signal which can be attributed to the glyceryl carbon at the ether function. According to model compounds found in the literature (19), this signal should appear in the region δ 70–72 ppm, but it was not present in the spectrum of MA-IN as was proved by the DEPT 135 spectrum (not shown). Assuming the same reaction mechanism as proposed by Vermeersch and Schacht for the synthesis of inulin monosuccinates (20), transesterification can be explained as follows: nucleophilic catalysis of DMAP, resulting in the intermediate formation of a methacryloyl pyridinium salt and general base catalysis results in the methacrylation of inulin. The reaction mechanism is schematically depicted in Fig. 4. Since the coupling reaction of GMA to inulin involves base catalysis, derivatization probably takes place at the C-6 hydroxyl of the fructose unit due to higher acidity of a primary alcohol compared to a secondary alcohol and also due to lower steric hindrance. Fig. 3A and Table I show that, in addition to the signals of the methacryloyl group and the inulin carbon atoms (21,22), several additional peaks are present. The signal at δ 65.16 ppm clearly originates from an O-esterified C-6 carbon that has been typically downfield shifted (3.60 ppm), confirmed by the negative signal in the DEPT 135 spectrum (not shown). Additionally, the signal at δ 78.46 ppm refers to the upfield shifted (3.20 ppm) C-5 carbon adjacent to the substituted C-6. The position of the other signals can be attributed to the influence that the other carbons experience from the substitution at C-6 (shifts of about 1 ppm or less). The signal at δ 4.44 ppm in the ^1H NMR spectrum of MA-IN (Fig. 2) can be attributed to the about 1 ppm downfield shifted protons at the derivatized C-6 carbons. O-methacrylation at C-3 (δ 4.25

ppm) and C-4 (δ 4.07 ppm) should result in a downfield shift of ca. 1.5 ppm (20) giving rise to signals at approximately δ 5.75 and δ 5.57 ppm. These signals are absent in the spectrum, indicating that derivatization at C-3 and C-4 does not occur.

For a proper characterization of MA-IN, the DS was calculated based on the assignment of the ^{1}H NMR spectrum and using equation 2:

$$DS = \frac{7 \cdot x}{y + z} \times 100 \qquad (2)$$

with x the average integral of the signals for the sp^2-protons at δ 5.78 ppm and δ 6.20 ppm, y the integral of the broad signal between δ 3.30–4.60 ppm assigned to the fructose and glucose protons and z the integral of the signal for the anomeric proton of glucose at δ 5.42 ppm.

In addition, the DS was also determined by titration. The results obtained by both analytical methods, are reported in Table II and agree rather well. It appears that the DS of MA-IN can be tuned by varying the molar ratio of GMA to inulin in the reaction mixture. The efficiency of the coupling reaction, calculated as the ratio of the obtained DS to the theoretical DS, seems to decrease with an increasing molar ratio of GMA to inulin.

Carbohydrate Analysis

In order to check whether the degree of polymerization of inulin was changed by the derivatization procedure, the individual oligomers and polymers of which inulin is composed, were examined before and after reaction using high- performance anion-exchange chromatography with pulsed amperometric detection. Fig. 5 shows the profile of the fructans of inulin and MA-IN DS 4.4. The initial polymer chain length was preserved during the whole derivatization procedure, indicating that no acid hydrolysis of the β-glycosidic linkages occurred upon addition of concentrated HCl to the reaction mixture.

Preparation of MA-IN Hydrogels

Hydrogels were obtained by free radical polymerization of aqueous solutions of MA-IN. During polymerization, the vinyl groups are converted into covalent inter- and intramolecular cross-links resulting in a three-dimensional network. Unfortunately, FT-IR spectroscopy could not be employed to follow the polymerization kinetics. The absorption at 1709 cm^{-1}, representing the carbonyl group, was clearly visible, but the twisting vibrations of the methacrylate double bonds in MA-IN, normally situated around 811 cm^{-1} (23,24), could not be distinguished from the inulin spectrum (spectra not shown). Hence, solid state ^{13}C NMR spectroscopy was used to demonstrate the conversion of the double bonds upon polymerization. The spectra of MA-IN (DS = 12.1) at the beginning and the end of the polymerization reaction are shown in Fig. 6. Upon polymerization, the carbonyl function next to the double bond, is shifted from δ 168.58 ppm to δ 178.39 ppm because of the loss of conjugation due to the change of the carbon, adjacent to the carbonyl function, from sp^2 to sp^3 (25). The vinyl carbon bearing the methyl group shifts from δ 136.18 ppm to δ 45.35 ppm upon changing from sp^2 to sp^3. Also the other vinyl carbon at δ 128.57 shifts upfield but the signal, expected in the region around 55 ppm, is overlapped by the sugar carbons. From this experiment it was however not possible to determine quantitatively the % conversion of the methacryloyl groups.

CONCLUSION

Incorporation of vinyl groups in the fructose chains of inulin was accomplished by reaction of inulin with GMA in DMF in the presence of DMAP as catalyst. In contrast to literature data, reaction did not take place at the epoxide ring of GMA, but transesterification occurred. ^{1}H and ^{13}C NMR spectroscopy experiments also revealed that the methacryloyl group was attached to the C-6 hydroxyl of the fructose units. Free radical polymerization of aqueous MA-IN solutions using APS and TMEDA as initiating system, converted the double bonds into covalent cross-links resulting in inulin hydrogels.

Characterization of the prepared inulin hydrogels (rheological and swelling behavior), their bacterial degradability and the release of drugs from these delivery systems will be dealt with in forthcoming publications.

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